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Experimental transplantation of skin preserved by sub-freezing to - 196°C in liquid nitrogen

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The preservation of tissues by sub-freezing prior to grafting is assuming an ever increasing importance in restorative surgery. Untill quite recently it was not too clear as to what extent and how long frozen tissues could maintain their viability and function following transplantation.

The tissues with static, supporting or framework function (such as bone, cartilage, blood vessels etc.) can be effective clinically in the transplantation of dead, killed or fixed grafts Jet for effective grafting of tissues with an active function (l.g. skin, erdocrine glands and, particularly, large whole organs) it is essential that they maintain good viability following preservation.

To evaluate the viability of tissues following sub-freezing to - 196°C, seventysix experiments were made on 33 rabits. harge fullthickness skin pieces were immersed in liquid, nitrogen and left there for various periods of time, ranging from 1 hour to more than 1 month. In some experiments protective substances were used (vaseline oil, 15-13% glycerine solutions). Prior to grafting, the skin was warmed rapidly in lukewarme Saline.

Control experiments with the transplantation of fresh (non-preserved) rabbit-skin autografts showed a true survival for months and even years.

In transplantation of non-preserved rabbit-skin homografts, necrosis and sloughing of the graft was always observed following the period of initial or apparent acceptance.

Rabbit skin preserved by freezing to - 196°C and banked in liquid nitrogen up to 23 days and longer may remain viable and following autografting it is as capable of true survival as fresh non-frozen skin. Hair grows anew on the persistently accepted shaven depigmented skin pieces though at first the hair is also depigmented later, pigmentation is partially restored, with the process beginning uniformly over the entire surface of the graft, and not merely from the edges. It would seem that the longer skin is preserved, even in the temperature of liquid nitrogen, the worse it is accepted.

Following the period of initial acceptance, rabbit-skin homografts, preserved by sub-freezing to - 196°C in liquid nitrogen, become necrotized and slough or are gradually resorbed. Yet, they are tolerated much longer than transplanted non-preserved homografts (they remained histologically viable for periods of up to 65 days following the transplantation instead of 2 or 3 weeks). In some experiments during the period of initial acceptance a more solid union and a better clinical appearance were observed as compared to frozen autografts.

All attempts to implant rabbit skin preserved by freeze-drying at - 70°C (with the equipment and in the conditions as used for lyophilization of blood vessels) ended in failure, both in auto- and homografting. In these conditions lyophilized skin did not remain viable, in contrast to skin grafts, which were more deeply frozen but not dried.

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THE STUDY OF SKIN HOMOGRAFTS ON A VASCULARIZED PEDICLE WITH TRANSFUSION OF CADAVER BLOOD FROM THE SAME DONNER

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Thesis

Until recently the skin used to be grafted without immediate reestablishment of circulation. With this method it is very difficult to watch the processes of vital activity in the graft at the time of its death. And the causes leading to the death of a graft are more easily detected during the very first hour of its death.

In experiments on dogs we studied the condition of the skin homografts after immediate reestablishment of the circulation in the grafted piece. We found the presternal skin to be the most convenient of all for reestablishment of circulation. Its arteries and beins were sutured to the cervical artery and vein in the recepient dog.

We were able to establish the following:

Arrest of circulation in the graft is the immediate cause of its death.

Circulation in the graft will stop as a result of three causes:

a) thrombosis at the site of the arterial suture

b) thrombosis at the site of the venous suture

c) compression by fibrous adhesion of the surgical wound in the vein which provided for the blood outflow from the gra

With circulation present union of the graft with the adjacent tissues would take per prima in 8-9 days. In such an event hair would grow normally on the transplanted skin.

Best results were observed by us when the skin was taken together with a piece of sternum from a lactating dog.

In a series of experiments along with the transplantation of skin with a vascularized pedicle from the dog donor we transfused his cadaveric blood in order to bring closer together the internal environment of the recipient with the tissues of the graft.

In transplantation of the entire organs from the cadaver to man transfusion of the cadaveruc blood of the donor ought to exert favourable influence on the "taking " of the organ.

ON THE TRANSPLANATION OF THE HEART

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T h e s i s

Our experience, extending over many years, has shown that a transplanted heart undergoes much fewer chenges as compared with the transplanted kidneys during the same period of time after the operation.

The most common complications of heart transplanation are - pericarditis, as a result of infection in the surgical wound and myocardial infarction of thrombo-embolic origin.

From the functional point of view the transplanted heart is able to 50% to 100% of work in ensuring blood circulation in the body, depending on the type of the operation used.

In our experiments the heart was transplanted either from a living dog, in this case it was removed according to original technique with its rhythmic activity intact, or else from a dog's cadaver 1-2 hours after death.

In experiments the cadaveric heart was resuscitated by restoring circulation in the body of the recipient.

Lately we have started transplanting hearts into the thoracic cavity having covered them previously with a plastic bag, which permits direct visual control of the transplanted heart's function.

We are now conducting preliminary investigations before transplanting the heart to man.

Human cadavers brought to us by ambulance are resuscitated by two methods:

a) direct cardiac massage with temporary clamping of the thoracic aorta and with controlled respiration.

b) with the aid of artificial circulation ensured by a "mechanical heart" and with controlled respiration.

Initially we intend to "switch in" the resuscitated heart and lungs temporarily, instead of the mechanical heart and lungs, which are now widely used in surgical practice.

Due consideration will have to be taken of blood groups and other factors between the donor's and the recipient's blood before the resuscitated heart and lungs can be "switched in".

Transfusion of the cadaveric blood from the same donor ought to contribute to the success of the operation.

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Destruction of tolerated skin heterografts by means of serum antibodies

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The destruction of tolerated skin heterografts (between two species of ducks - Cairina moschata and Anas platyrhinchos) by passively transferred serum antibodies (immune sera were prepared by mutual immunization of adult individuals of both species with skin grafts and tissue homogenates in complete Freund's adjuvant) can be accomplished by intraperitoneal injections of sufficient quantity of immune sera. The serum was filtrated through Selas filter, neither freezing nor 30 minute heating at 56° C influenced the effectiveness of the serum. The irreversible complete destruction of skin graft is induced by a quantity of serum 8% higher the body weight of the recipient with the actual titre of the agglutinin 1:32 to 1:124. The reaction follows at a relatively rapid rate without the participation of hypersensitivity of delayed type. Two hours after transfer of serum, the tolerated graft turns haemorrhagic macroscopically and an oedema starts developing. During the next two days the graft is getting completely destroyed. Histological picture shows striking haemorrhagies, dilatated and thrombotized blood vessels, eosinophilic infiltration and poorly stained epidermis as soon as 2-5 hours after transfer and developed at the maximum of 12-24 hours. Non-immune sera of the opposite species have no similar effect. Thus far, we have only investigated the effect of the serum absorbed with erythrocytes. The antiserum from which the agglutinins had been removed is equally effective as a non-absorbed serum. The vascular-necrotic component in this reaction, quite similar to the passive Arthus phenomenon, is apparent in this model and also the cytotoxic antibodies with the greatest probability play a role in this reaction. A general significance of this model is discussed also in relation to homograft reaction.

Immunologic Potencialities of the Small Lymphocytes as revealed by Homotransplantation Experiments in Diffusion Chambers.

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Small lymphocytes were separated by gradient centrifugation and micromanipulation from a lymph cell suspension obtained from cisterna chyli of normal adult rabbits. The suspension of small lymphocytes was transferred in diffusion chambers into the peritoneal cavity of newborn rabbits. After 8-12 day cultivation it was found that approximately 6-10% of the original cells display potenciality of modulation into other cellular types. The transitional forms of the small lymphocyte were marked by enlarged nucleoli and high content of mitochondria. About 0,5% of the original cells became medium or even large lymphocytes displaying mitotic activity. About 1,5% of the original cells became reactive form of lymphocyte (Downey's type II lymphocyte). Another 3% became typical histiocytes and about 0,2% plasmacytoid elements. These changes are regarded as to be due partly to the nonspecific irritation inside the diffusion chambers and partly to the effect of the homologous peritoneal fluid forming the cultivation medium. These changes, especially the formation of plasmacytoid cells, were enhanced by addition of protein antigens. The cell suspensions with protein antigens produced antibodies in low titers. Antibodies could be found by Coons technique in plasmacytoid cells and occasionally also in reactive forms of lymphocytes.

Cultivation of lymph cell suspensions with normal or enlarged content of medium and large lymphocytes gave under the same conditions also typical plasma cell differentiation and a higher antibody formation.

A Contribution to the Problem of Long-Term Release of Transplantation Antigens from Cutaneous Homografts.

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In one of our previous investigations we succeeded to obtain a permanent survival of cutaneous homografts on a rabbit ear in 3 out of 14 cases by affecting the regional lymphatic system of the host by the transplantation of a node immunized against the host (Vrubel 1961). We are aware of the fact that this effect is extremely difficult to interprete. In our conception this activity is interpreted as a definite and shortly limited destruction of the lymph of the host cells produced by the action of antibodies which are directed against the host tissue. This led us to study the length of time during which the transplantation antigens are released from the graft.

For this purpose use was made of the antigen-antibody reaction in the lymphatic system of the rabbit ear using the concurrent transfer both of a cutaneous homograft and a suspension of lymph node cells immunized against this graft. An extensive oedema developed in this ear within 24 to 48 hours and its degree was estimated by weighing the ear 48 hours after the transfer. In the first series of animals a node immunized against the graft was transferred to the corresponding ear of the rabbit in varying intervals after the skin transplantation. It has been found that practically no oedema developed after an interval as short as 5 days between the transplantation of skin and the immunized node.

It is likely that the immunity of the host against the graft develops at that time already and that antigens may have been destroyed by the antibodies of the host so that the latter do not necessarily penetrate into the lymphatic system of the ear and react with antibodies against the graft, transferred adoptively.

This is why in another trial series the regional lymph nodes of the host were extirpated. Under this condition the survival of the graft is about twice as long (Vrubel, Stark, Dwyer) and the development oedema as result of the interaction between the antigen and antibody remains unaffected. In this trial series it was found that no oedema developed in the ear in the 7-day interval elapsed between the transplantation of the graft and the node immunized against the former.

These experiments appear to suggest that the transplantation antigens are released immediately after transplantation and that the process lasts for a relatively short time being probably dependent on the healing process of the graft.

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Successfully homotransplanted kidneys in dogs.

A.Gombos, V.Tischler, J. Jacina, J. Skoken

We had shown that specific immunologic tolerance could be induced in puppies/mongrel/ subjected to complete blood exchange in the first days of their life with blood from the prospective donor. Subsequent homotransplantation of kidneys from blood donors was performed in 15 animals. Although the method of exsanguination-transfusion and the technic of kidney grafting was the same in all cases, only in 5 dogs kidney transplantation have been successful. Successful kidney homografts in 3 dogs continue to function on the 19 months and on the 16 months /2 dogs/.

Elimination of the host-vs.-graft reaction is sufficient to performe successful homotransplantation of kidneys, and that the graft-vs.-host reaction is inconsequential /if it exists/ when tolerance has been induced in the host by prior exsanguination- transfusion. We found no evidence of a graft-vs.-host reaction.Successful kidney homografts show no cell infiltration - typically present when the exchange of blood has not induced immunologic tolerance.

The general reaction of the kidney graft recipient predicts accurately the presence or absence of immunologic tolerance.

Rejection of a skin homotransplant in dogs with exsanguination- transfusion cannot always serve as a reliable prediction of the rejection of a subsequent kidney graft from the same donor. (Certainly in the more usual situation, when a skin graft is

successful, a kidney transplant from the same donor will also be accepted, and when the skin graft is rejected, the kidney transplant will be rejected).

Our long-term observation of the function (C_{in}, C_{PAH}, FF, R of water, renal elimination of inorganic phosphate, citric acid etc.) of homotransplanted kidneys in immunologically tolerant dogs show that these kidneys fulfill their homeostatic function when the host has only the transplanted kidney (more than one year). Following nephrectomy of both the animal's own kidneys, we observed that a single homografted kidney functionaly hypertrophised.

Successful kidney homotransplants behave like the kidney autotransplants.

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EIGHTY PERCENT BODY SURFACE BURN TREATED BY MASSIVE HONOGRAFT PROCEDURES

The routine application of skin homografts in the treatment of extensive burns offers good opportunities for the clinical observation of the behaviour of homografts, from the moment of their application to their elimination.

Valuable data in this regard was provided by an exceptionally severe case of a nine-year old boy with an 80 percent body surface burn.

The treatment of such a case in which homotransplantation figures prominently, is complicated by the /a/ extensiveness of the burnt surface, /b/ the circumference of the burnt areas, and /c/ the problem of skin-donors.

In the above mentioned case, homografts from several donors were applied at given intervals, in the following order: first, skin grafts taken from the boy's mother were used to cover about 35 percent of the body surface /almost the whole of the posterior part of the body/. Eight days later, the front part of the body was covered with homografts taken from the father. As the homografts taken from the mother and father were eliminated, a new set of grafts from 12 skin donors at two intervals were applied. Homografts from a cadaver were used in the final stage.

The homografts taken from the mother had the longest survival time - eight weeks. The homografts from the father and from both groups of six skin-donors each sloughed after three weeks, while the cadaver homografts

were eliminated after two weeks. In all the phases, the take of the homografts was 100 percent.

The clinical criterion alone was used in determining the survival time of the homografts, e.g., the firmness of adherence of the graft to the host and its general appearance.

Several factors were found to be of particular importance in regard to the "take" of the homografts and their survival.

First, immunotransfusion of blood and plasma. This measure which was life-saving in the stage of severe toxaemia in this case, also had a remarkable effect in keeping the granulating surfaces healthy. In particular, it had an excellent effect on the patient's general condition and its constant improvement.

Second, repeated transplantations from multiple donors. The results obtained through the application of this method seem to confirm the views to the effect that there are advantages in using grafts from many donors instead of from a single donor.

Third, the exposure of homografts without dressings. This technical detail is important because the grafts are, in this way, protected from possible disturbances by the dressings, the milieu is kept dry and there is a better possibility for the direct supervision of the grafts.

Finally, the alternated application of large-size homografts and autografts. In our case, this method was found to be more effective than the use of small-size grafts which many authors recommend.

In comparing this case with other cases of extensive burns, which were treated by the usual techniques without immunotransfusion, we were able to conclude that the above-mentioned measures definitely aided the "take" of the homografts and prolonged their survival time.

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Abstract of the paper:

THE CONCEPT OF COMPETITIVE REPLACEMENT

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Three experimental models have been described by which specific tolerance to living tissue can be induced. First, by injection of cells from prospective homograft donors into immunologically immature animals /1/; second, by injection of bone marrow into lethally irradiated animals /2/; and third, by parabiosis of embryos /3/ or adult animals /4/.

Parabiosis of adult animals differs from other experimental models in that tolerance is induced in immunologically competent partners and that an already sensitized parabiont is subsequently rendered specifically unresponsive /5/.

The competitive replacement of the immune system of the host by that of the donor has been proposed as a possible mechanism responsible for induction of tolerance in adult rats by short-term parabiosis /6/. Following exchange of immunologically competent cells between parabiotic partners as soon as the vascular anastomoses open up, the replacement would take place as a result of competition of actively immunized cells of the donor and the host and the consecutive extermination of host lymphoid cells in situations where antigenic conditions favour a stronger unidirectional immunity reaction.

The replacement of the immune system might be a mechanism whereby specific tolerance is maintained in lethally irradiated animals treated with homologous or heterologous bone marrow.

The same mechanism might also be responsible for the phenomenon

of acquired tolerance in the sense of Billingham, Brent and Medawar /1/. The concept of competitive replacement might explain the following facts not satisfactorily accounted for by the theory of acquired tolerance:

1. Specific tolerance to living tissue in the so called 'difficult systems' can be induced only with immunologically competent cells.

2. Cell-free material is largely ineffective in inducing tolerance to living tissue /7/.

3. It is difficult to induce tolerance with adult F_1 spleen cells in new-born mice of parental strain (8,9).

4. The irradiated spleen cells are unable to provoke a tolerant state of even a few days duration /1o/.

5. In many strain combinations it is easier to induce tolerance in one direction than in the other (e.g. C57 → A, CBA → A, C3H → A /8/, Lewis → B.N. /11/) and the direction seems to depend on the difference in the number of antigenic components between the donor and the host (see Gorer /12/).

References

1. Billingham,R.E.,L.Brent and P.B.Medawar, Nature,172:6o3,1953
2. Main,J.M. and Prehn,R.T., J.Nat.Cancer Inst.,15:1o23,1955
3. Hašek,M., Čs.Biologie,2:265,1953
4. Nakić,B. and V.Silobrčić, Nature,182:264,1958
5. Nakić, B., Ciba Foundation Symposium on Transplantation, 1961
6. Nakić,B. et al., Brit.J.Exp.Path.,42:63,1961
7. Billingham,R.E.,L.Brent and P.B.Medawar, Transpl.Bull.,5:377,1958
8. Billingham,R.E. and L.Brent, Phil.Trans.B,242:439,1959
9. Woodruff,M.F.A. and D.Michie, Nature (in the press)
1o. Howard,J.G. and D.Michie,Transpl.Bull.,27:455,1961
11. Billingham,R.E. et al., Ann.N.Y.Acad.Sci.,87:462,196o
12. Gorer,P.A., Les Congrès et Colloques de l'Université de Liège, vol.12, p.25,1959